ELEVATION AND RETRACTION OF THE FERTILIZATION MEMBRANE OF ECHINODERM EGGS FERTILIZED IN PAPAIN SOLUTIONS[1]

ALBERT TYLER AND MELVIN SPIEGEL[2]

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

It has been shown by Runnström *et al.*, (1943), Moore (1949) and others that treatment of unfertilized eggs of sea urchins with solutions of trypsin or chymotrypsin causes the eggs to fail to elevate a membrane upon fertilization. Normal development can ensue after such treatment, although the quantity of sperm required for successful fertilization is found (Tyler and Metz, 1956) to be greater than for untreated eggs. The treated eggs are also more susceptible to polyspermy (Hagström and Hagström, 1954) and more susceptible to cross-fertilization (Hultin, 1948; Bohus-Jensen, 1953; Tyler and Metz, 1956).

The action of trypsin in causing failure of membrane-elevation upon fertilization has been interpreted (Runnström *et al.*, 1943; Minganti, 1953) as due to a dissolution of the vitelline membrane of the unfertilized egg, which, together with material of exploded cortical granules, is considered (Motomura, 1941; Runnström *et al.*, 1944) to be the precursor of the fertilization membrane. Doubts have, however, been expressed (Mitchison and Swann, 1952; Hillier *et al.*, 1952) as to the separate existence of a vitelline membrane and of its dissolution by proteolytic enzymes (see also Tyler and Metz, 1956). The examination of the egg surface by polarization-microscopy (Swann and Mitchison, 1951; Mitchison and Swann, 1952) and by electron microscopy (Hillier *et al.*, 1952; see, however, McCulloch, 1952) revealed no distinct structure corresponding to the vitelline membrane. Runnström *et al.* (1943) showed that trypsin-treated eggs behave differently from untreated eggs upon shrinking and swelling in hypertonic and hypotonic solutions, which could be interpreted as due to loss of the vitelline membrane. It is possible, however, that the difference might be due to an alteration of the surface by combination with trypsin rather than the removal of anything therefrom. Parpart (personal communication) examined trypsin-treated eggs of *Arbacia* under the "television microscope" and could observe no change in the appearance of the vitelline membrane.

There are also cases, notably in the sand dollar *Dendraster excentricus* (Moore, 1951) and in the heart urchin *Echinocardium cordatum* (Runnström, 1948), in which treatment of the unfertilized egg with trypsin causes an elevation of a membrane.

In the present experiment the action of the enzyme papain was examined during the process of fertilization and it was observed that membrane elevation could occur

[1] This work was supported by a grant (C-2302) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

[2] Fellow of the National Institutes of Health, Public Health Service. Present address: Department of Biology, Colby College, Waterville, Maine.

in the presence of the enzyme but that the membrane subsequently returned to the surface of the egg.

Material and Methods

Eggs of the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* and of the starfish *Asterias forbesii* were used in this work.

The papain solution was prepared by extracting for several hours in the cold two grams of papain (Nutritional Biochemical Corporation) with 100 ml. of sea water containing 10^{-4} moles of Versene [3] (ethylenediaminetetraacetic acid). The Versene was included because of its effect of improving the fertilizing capacity of the sperm (Tyler, 1953). In some experiments the Versene was omitted. The extract (termed a 2% solution) was filtered and diluted (with 10^{-3} *M* Versene or with sea water) to the concentration desired. To this, just before use, cysteine hydrochloride was added to give a concentration of 0.2% and the pH adjusted to between 8.1 and 8.2.

Results

When unfertilized eggs of *Strongylocentrotus* or of *Lytechinus* were treated with solutions of papain ranging from 0.01 to 2% for periods of 5 seconds to one hour, and then inseminated in the solution, fertilization ensued without the elevation of a fertilization membrane. Such eggs cleaved while in the papain solution, the blastomeres separating from one another. When the unfertilized eggs were washed with sea water after treatment with papain solution (0.01% to 2% for periods of five seconds to one hour) they similarly failed to elevate a membrane upon fertilization. Cleavage of such eggs was normal and normal plutei developed.

When unfertilized eggs were added to a suspension of sperm in a 0.04% or 0.02% solution of papain, membrane elevation occurred. However, the membrane subsequently retracted to the surface of the egg. Figure 1 illustrates the course of this phenomenon in eggs of *Strongylocentrotus*. In this series eggs were added to a 0.1% suspension of semen in a 0.04% solution of papain. The membrane is observed to be fully raised after 1½ minutes (Fig. 1c). From then on it progressively shrinks until at 5½ minutes it has returned to the egg's surface, from which it is no longer distinguishable. If left in the solution the blastomeres tend to separate upon cleavage. If removed from the solution at 5½ to 20 minutes cleavage is normal with a distinct ectoplasmic (hyaline) layer visible, but no indication of a fertilization membrane (see Fig. 1, 1). One hundred per cent normal cleavage can be obtained in this manner and later development is as good as in the control eggs.

Similar results were obtained with the eggs of *Lytechinus* and those of *Asterias*. In the latter the membrane elevates completely at 5 minutes and retracts to the surface (no longer distinctly visible) at 20–30 minutes after insemination.

Discussion

The main point of the present paper is that a fertilization membrane can be elevated and then retracted, as described above, during the first few minutes after insemination. There is no indication of the presence of this membrane as a dis-

[3] Supplied by Bersworth Chemical Corporation, Framingham, Mass.

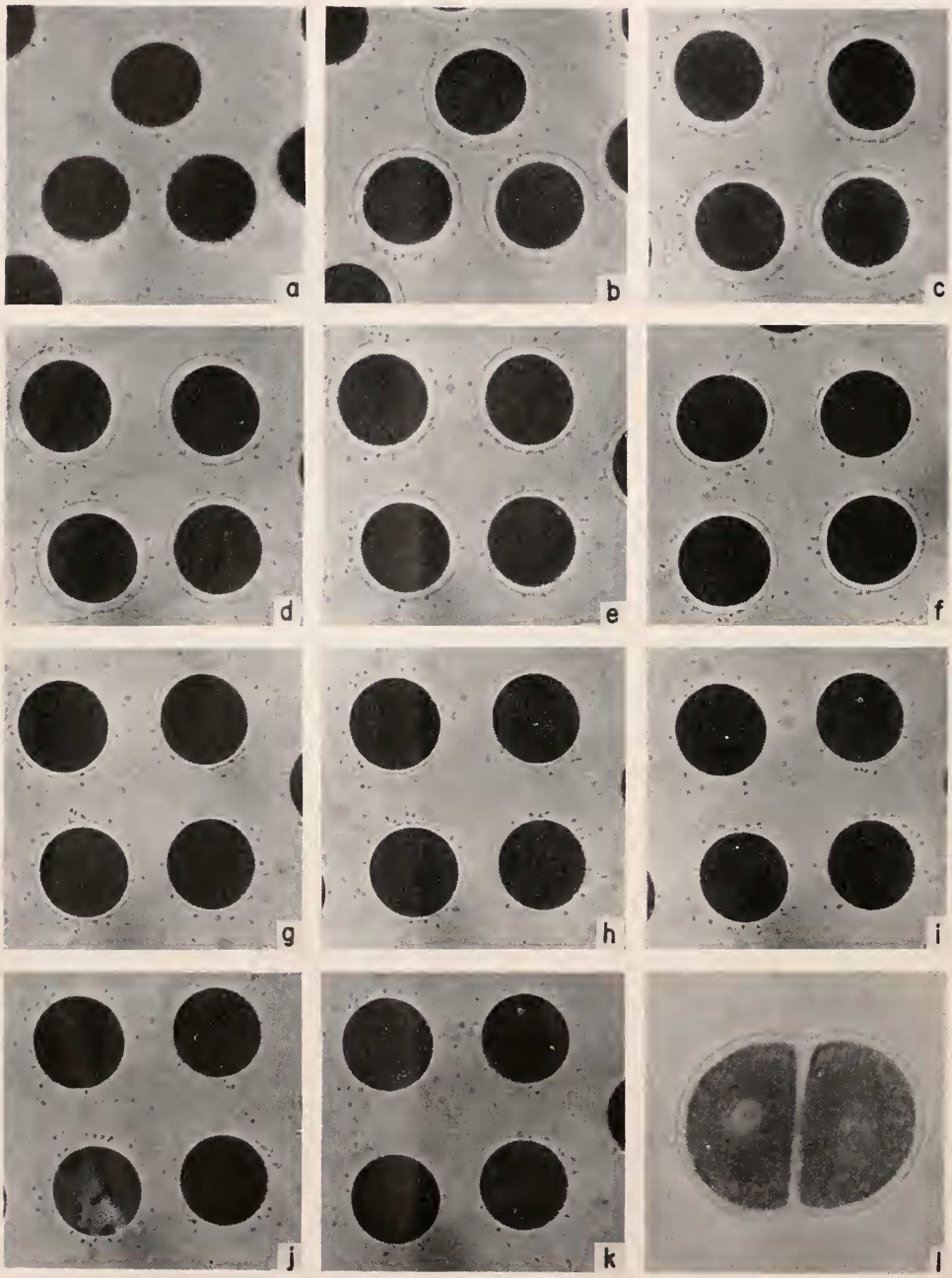

FIGURE 1. Papain demembranation of *Strongylocentrotus purpuratus* eggs. One drop sperm (in 0.02% papain) mixed with one drop egg suspension on slide sealed with vaseline. Figure (a) taken 35 seconds after eggs added, figures (b)–(k) taken at 30-second intervals. Figure 1 (l) shows a papain-treated egg (removed to sea water at 15 minutes) in the 2-cell stage. Magnification: Figures (a)–(k), 147 ×; figure (l), 333 ×.

tinct entity during subsequent development of the egg. This may be interpreted to mean that further enzyme-action after the membrane has reached the egg's surface results in its dissolution. It should be noted, however, that when eggs of sea urchins are treated with proteolytic enzymes after the fertilization membrane has fully formed (a few minutes after fertilization) the latter is not visibly affected (see Moore, 1949; Runnström *et al.*, 1943; Minganti, 1953). The same is true for treatment with papain solutions. This, then, does not support the interpretation that there has been a dissolution of the membrane by enzymatic action after return to the surface. Our present interpretation is that the contracted membrane fuses with the egg surface and its material remains a part thereof during subsequent development.

Summary

When eggs of sea urchins and starfish are added to a suspension of sperm in papain solutions (0.02 to 0.04%), a fertilization membrane is elevated in the normal time and, after reaching full separation, retracts to the surface of the egg. This process is completed in approximately 5 minutes in the case of sea urchins (*Strongylocentrotus purpuratus* and *Lytechinus pictus*) and 25 minutes in the case of starfish (*Asterias forbesii*). It is not visible during subsequent development after removal of the eggs from the solution, and development to late stages is normal. Treatment of unfertilized eggs with papain prevents membrane elevation upon fertilization, as reported for other proteolytic enzymes. Treatment of eggs with fully formed fertilization membranes shows no effect on these. It is suggested that the retracted membrane, obtained by fertilization in papain solution, fuses with the egg surface, and that, in general, the question remains open as to whether or not proteolytic enzymes dissolve the vitelline membrane of the unfertilized egg.

LITERATURE CITED

Bohus-Jensen, A., 1953. The effect of trypsin on the cross-fertilizability of sea urchin eggs. *Exp. Cell Res.*, **5**: 325–328.

Hagström, B., and B. Hagström, 1954. The action of trypsin and chymotrypsin on the sea urchin egg. *Exp. Cell Res.*, **6**: 532–534.

Hillier, J., A. I. Lansing and T. B. Rosenthal, 1952. Electron microscopy of some marine egg surfaces. *Biol. Bull.*, **103**: 293.

Hultin, T., 1948. Species specificity in fertilization reaction. I. The role of the vitelline membrane of sea-urchin eggs in species specificity. *Arkiv. f. Zool.*, **40A**, 12: 1–9.

McCulloch, D., 1952. Note on the origin of the cortical granules in *Arbacia punctulata* eggs. *Exp. Cell Res.*, **3**: 605.

Minganti, A., 1953. The action of proteolytic enzymes on the vitelline membrane of *Psammechinus miliaris* eggs. *Exp. Cell Res.*, **5**: 492–499.

Mitchison, J. M., and M. M. Swann, 1952. Optical changes in the membranes of the sea urchin egg at fertilization, mitosis and cleavage. *J. Exp. Biol.*, **29**: 357–362.

Moore, A. R., 1949. The relation of ions to the appearance of the fertilization and hyaline membranes in the eggs of the sea urchin. *Amer. Nat.*, **83**: 233–247.

Moore, A. R., 1951. Action of trypsin on the eggs of *Dendraster excentricus*. *Exp. Cell Res.*, **2**: 284–287.

Motomura, I., 1941. Materials of the fertilization membrane in the eggs of echinoderms. *Sci. Rep. Tohoku Imperial University, 4th Series Biology*, **16** (3): 345–363.

Runnström, J., 1948. On the action of trypsin and chymotrypsin on the unfertilized sea-urchin egg. A study concerning the mechanism of formation of the fertilization membrane. *Arkiv. f. Zool.*, **40A** (17): 1–16.

RUNNSTRÖM, J., L. MONNÉ AND L. BROMAN, 1943. On some properties of the surface layers in the sea urchin egg and their changes upon activation. *Archiv. f. Zool.*, **35A** (3) : 1–32.

RUNNSTRÖM, J., L. MONNÉ AND E. WICKLUND, 1944. Mechanism of formation of the fertilization membrane in the sea urchin egg. *Nature*, **153** : 313–314.

SWANN, M. M., AND J. M. MITCHISON, 1951. Birefringence of cytoplasm and cell membranes. *Progress in Biophysics*, **2** : 1–16.

TYLER, A., 1953. Prolongation of life span of sea urchin spermatozoa, and improvement of the fertilization reaction, by treatment of spermatozoa and eggs with metal-chelating agents (amino acids, Versene, DEDTC, oxine, cupron). *Biol. Bull.*, **104** : 224–239.

TYLER, A., AND C. B. METZ, 1956. Effects of fertilizin-treatment of sperm and trypsin-treatment of eggs on homologous and cross-fertilization in sea-urchins. *Pubbl. Staz. Zool. Napoli*, in press.